Breast cancer risk factors according to joint estrogen receptor and progesterone receptor status

Jennifer A. Rusiecki PhD[a],[*], Theodore R. Holford PhD[a],
Shelia H. Zahm PhD[b], Tongzhang Zheng DSc[a]

[a] Department of Epidemiology and Public Health, Yale University, School of Medicine, New Haven, CT, USA
[b] Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, DHHS, Bethesda, MD, USA

Abstract

Background: We investigated risk factor patterns for subtypes of breast cancer characterized by joint estrogen receptor (ER) and progesterone receptor (PR) status in a hospital-based case-control study. *Methods:* ER and PR tumor status were determined immunohisotchemically. Risk factors of interest were entered into a multiple polychotomous logistic regression model simultaneously; odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Using this model, cases in the four tumor subtypes (ER$^+$PR$^+$, ER$^-$PR$^-$, ER$^+$PR$^-$, ER$^-$PR$^+$) were compared simultaneously to controls. A Wald test for heterogeneity across the four subtypes was conducted, as well as a case–case comparison between the two most biologically disparate subtypes, ER$^+$PR$^+$ and ER$^-$PR$^-$. *Results:* The receptor status distribution was as follows: 33% ER$^+$PR$^+$, 34% ER$^-$PR$^-$, 20% ER$^+$PR$^-$, and 13% ER$^-$PR$^+$. Among 317 cases and 401 controls, we found significant heterogeneity across the four tumor subtypes for older age at first full-term pregnancy ($p = 0.04$) and post-menopausal status ($p = 0.04$). For older age at first full-term pregnancy, an elevated risk was found for the ER$^+$PR$^-$ subtype (OR = 2.5; 95% CI: 1.2–5.1). For post-menopausal status, elevated risks were found for both the ER$^+$PR$^+$ (OR = 2.4; 95% CI: 1.1–4.9) and ER$^+$PR$^-$ (OR = 7.2; 95% CI: 2.4–21.7) subtypes. From the case–case comparisons, we found that cases, who had consumed alcohol for more than 1 year were 3.4 times more likely to have ER$^+$PR$^+$ tumors than ER$^-$PR$^-$ tumors (95% CI: 1.4–8.4). *Conclusions:* Certain breast cancer risk factors may vary by ER and PR status, and joint ER/PR status should be taken into account in future studies of risk factor estimates.

Keywords: Breast cancer; Risk factor; Estrogen receptor; Progesterone receptor; Hormone; Age; Menarche; Lactation; Menopausal status; BMI; Alcohol intake; Smoking; Family history; Race

1. Introduction

It is well established that a woman's reproductive history influences her risk of breast cancer and that certain hormone-related risk factors are associated with an elevated risk. These include age at menarche, parity, age at first full-term pregnancy, lactation history, menopausal status, and age at menopause [1,2]. The longer the estrogen exposure, or the later in life the pregnancy-induced estrogen elevation occurs, the higher the breast cancer risk [1,2]. Breast cancer risk has also been found to increase with alcohol consumption and elevated BMI (particularly among post-menopausal women), potentially via an effect on sex hormone levels [3]. However, most of these well-established risk factors have been found to have modest relative risks and account for only about 20–40% of all breast cancer cases [4–6]. Additionally, the major known genetic risk factor, having a mutation in the BRCA1 or BRCA2 genes, may account for only about 7% of cases [7]. One possible explanation for these week relative risks and low attributable risks is that risk may vary across different groups of clinically and biologically distinct breast cancers [8], and analyzing breast cancer as one disease may obscure associations with these risk factors.

* Corresponding author. Present address: Occupational Epidemiology Branch, National Cancer Institute, NIH, DHHS, 6120 Executive Blvd., EPS 8111, MSC 7240, Bethesda, MD 20892-7240, USA. Tel.: +1 301 435 2358; fax: +1 301 402 1819.

E-mail address: rusieckj@mail.nih.gov (J.A. Rusiecki).

The hormonal status of a breast tumor has been used to predict a patient's response to endocrine therapy. Historically, ER status alone was used, however, it has been found that the predictive power is enhanced when both ER and PR status are considered jointly [8]. In addition, a gradient of responsiveness to endocrine therapy and of survival according to joint ER/PR status has been detected [9–13], in that the majority of women with ER$^+$PR$^+$ tumors respond favorably to endocrine therapy, while about a third with ER$^+$PR$^-$ and only 10% with ER$^-$PR$^-$ tumors respond favorably [13]. These findings suggest that ER/PR status might represent different disease entities of breast carcinoma [14–16]. Based on these findings, it has been hypothesized that tumors responsive to both hormones (ER$^+$PR$^+$) may be more closely associated with hormonally mediated risk factors, tumors unresponsive to both hormones (ER$^-$PR$^-$) may be inversely associated with hormonally mediated risk factors and more closely related to non-hormonally mediated risk factors, and hormone receptor discordant tumors (ER$^+$PR$^-$ and ER$^-$PR$^+$) may show an intermediate effect, thus a *gradient* of effect.

Epidemiologic studies that have examined breast cancer risk factors by either ER [15–29] or PR [16,23,26,29] status separately have generally shown moderate, inconsistent and non-significant associations. This could be in part because stratifying cases on ER or PR status alone obscures associations revealed by considering their joint effects. Only a few epidemiologic studies examining multiple breast cancer risk factors have classified cases by joint status of ER and PR [8,30–35]. Another few studies examined one or two risk factors by joint ER/PR status, e.g., family history [14], body size and physical activity [36], alcohol intake [37], body weight [38], dietary fat intake [39], and hormone replacement therapy [40]. The body of literature suggests that breast cancers defined by joint hormone receptor status may be distinct and that risk factors may vary by joint receptor status. However, with the exception of early age at menarche and post-menopausal obesity being associated with increased risk of ER$^+$PR$^+$ tumors, there has not been a strong consistency in the patterns of association found across the four tumor subtypes [41]. Additionally, as pointed out in a recent review of this literature, additional studies are now required to elucidate the differences in breast cancer risk factors by receptor status, and formal tests should be done to determine whether these groups are different with respect to the magnitude and direction of the relationship [41].

Based on the biological and clinical distinctions among the different tumor subtypes stratified by joint ER/PR status, we analyzed data from a hospital-based case-control study in Connecticut to investigate the patterns of associations for well-established breast cancer risk factors across the four subtypes. All measurements for ER and PR tumor status were done immunohistochemically, in the same laboratory, and there is comprehensive data for each study subject regarding reproductive, behavioral, family history and demographic risk factors.

2. Materials and methods

2.1. Study subjects

Data were collected from women who had breast-related surgery at Yale, New Haven Hospital (YNHH) between 01 January 1994 and 30 December 1997 and who were between the ages of 40 and 80 years. The general characteristics of the study population and the data collection procedures have been described in detail elsewhere [42]. Briefly, the study pathologist classified the potential participants as either cases or controls. Cases were histologically confirmed, incident breast cancer patients. Controls were patients without breast cancer who had histologically confirmed normal tissue, non-proliferative benign breast disease, or incident fibroadenoma. Patients diagnosed with atypical hyperplasia were excluded from the study. Informed consent was obtained from all potentially eligible participants.

Potentially eligible cases and controls from YNHH were identified using computerized patient information from the YNHH Surgical Pathology Department. All breast cancer patients who met the study eligibility requirements as described above were consecutively entered into the study. Controls eligible for the study were randomly selected. Cases and controls had no previous diagnosis of cancer, with the exception of non-melanoma skin cancer, and were alive at the time of interview. Efforts were made to frequency match the cases and controls by age within 5 year intervals (e.g., 40–44, 45–49, 50–54 years) with a ratio of 1:1, by adjusting the number of controls randomly selected in each age stratum, every few months. The response rates were 71% for controls and 77% for cases.

ER and PR levels were measured immunohistochemically at the Pathology Department of YNHH. Both ER and PR status were considered positive when their H-score was higher than 75, as described by McCarty et al. [43]. An H-score (a measure of the relative quantity of protein) of 75 from histochemical localization is considered the equivalent of 20 fmol (femtomoles)/mg of protein for the biochemical analyses using dextran-coated charcoal [43]. Of the 420 cases, 318 had known receptor status.

After approval by each subject's physician, potential participants were interviewed by a trained interviewer, using a standardized, structured questionnaire to obtain information on well-established risk factors [42]. Breast cancer risk factors we analyzed were age at diagnosis ($\leq$50, >50 years), age at menarche (<12, 12–13, $\geq$14 years), age at first full-term birth (or stillbirth)/nulliparity (<30, $\geq$30 years, nulliparous (women who never gave birth, even if ever pregnant)), lifetime lactation ($\geq$12, 1–11 months, never), menopausal status (pre, post (based on whether a woman was still having menstrual periods, not including those due to estrogen replacement therapy)), body mass index (BMI (kg/m^2): <25, 25–29.99, $\geq$30), ever use of exogenous estrogen (at least 1 month use of estrogen reported, either as oral contraceptive or hormone replacement therapy), alcohol

intake (<1 year, ≥1 year intake of beer, wine, liquor, combined), smoking (never, 1–10, >10 pack-years), family history of breast cancer in a first degree relative (yes, no), and race (White, non-White). The participants in this study were predominantly White (88%), and the category of non-White included Black (9%), Asian (1%), and ''Other'' (2%) women.

2.2. Data analysis

All cases with known and unknown receptor status were compared to controls via unconditional logistic regression, in order to estimate the magnitude and significance of each risk factor, which was simultaneously entered into the model. To investigate differences by joint ER/PR status, we excluded cases with 'unknown' hormone receptor status and compared the four ER/PR subtype cases (ER$^+$PR$^+$, ER$^-$PR$^-$, ER$^+$PR$^-$, ER$^-$PR$^+$) simultaneously to controls in a multiple polychotomous logistic regression (MPLR) model, in which all risk factors were entered into the model. This model enabled simultaneous odds ratio and 95% confidence interval (CI) estimation for breast cancers of differing receptor status and a given risk factor with respect to the common control group. For each risk factor, a Wald statistic was calculated to determine the p-value (based on a Chi-squared test) for heterogeneity among the four case subtypes, providing us a formal test to determine whether or not risk varied by ER/PR status. In addition, we derived ORs and 95% CIs from ER$^+$PR$^+$ to ER$^-$PR$^-$ case comparisons to quantify the difference (estimate the heterogeneity) in risk between these two subtypes, because clinical evidence has shown them to have the greatest biological difference among the four joint tumor receptor subtypes.

All odds ratios in this study were calculated using unconditional logistic regression and MPLR in the SAS program, LOGISTIC [44].

3. Results

When all cases (with known or unknown ER/PR status; n = 420) were compared to controls (n = 406) in a model where all risk factors of interest were entered simultaneously (Table 1), there was a significantly increased OR for post-menopausal status (odds ratio (OR) = 1.8; 95% CI: 1.2–2.8); of the 826 women included in this analysis, 575 (70%) were post-menopausal. There was a significantly decreased OR for ever use of estrogen (OR = 0.6; 95% CI: 0.4–0.9); 25% of women in the study reported ever use of estrogen. A non-significant, monotonic increase was found for decreasing age at first menstrual cycle. None of the other recognized or suspected risk factors showed a significant association with breast cancer.

Estrogen receptor and progesterone receptor status was known for 318 (75%) of the 420 cases. For women with known receptor status, 33% were ER$^+$PR$^+$, 34% were

Table 1
Adjusted odds ratios[a] for breast cancer among 420 cases and 406 controls, Yale, New Haven Hospital, Connecticut, 1994–1997

Risk Factor	Cases (n = 420)		Controls (n = 406)		OR (95% CI)
	No.	%	No.	%	
Age (years)[b]					
≤50	141	34	179	44	1.0
>50	279	66	227	56	1.2 (0.8–1.8)
Age at menarche (years)					
≥14	37	9	46	11	1.0
12–13	175	42	174	43	1.3 (0.8–2.2)
<12	208	49	186	46	1.4 (0.9–2.3)
Nulliparity/age (years) at 1st full-term pregnancy					
<30	303	72	268	66	1.0
≥30	62	15	63	16	1.0 (0.7–1.5)
Nulliparous	54	13	74	18	0.7 (0.4–1.0)
Unknown	1	1			
Lifetime lactation (months)					
≥12	70	17	68	17	1.0
1–11	82	20	88	22	0.8 (0.5–1.3)
Never lactated	268	63	250	61	1.0 (0.6–1.5)
Menopausal status					
Pre	102	24	149	37	1.0
Post	318	76	257	63	1.8 (1.2–2.8)
BMI (kg/m^2)					
<25.0	225	54	227	56	1.0
25.0–29.99	115	27	108	27	0.9 (0.7–1.3)
≥30	80	19	71	17	1.0 (0.6–1.4)
Ever use of estrogen					
Never	321	77	296	73	1.0
Ever	98	23	108	27	0.6 (0.4–0.9)
Alcohol intake (years)					
<1	64	15	63	16	1.0
≥1	356	85	343	84	1.1 (0.7–1.6)
Smoking (pack years)					
Never smoked	183	44	187	46	1.0
1–10 pack years	98	23	105	26	1.0 (0.7–1.4)
>10 pack years	139	33	114	38	1.2 (0.9–1.7)
Family breast cancer history					
No	318	76	319	79	1.0
Yes	102	24	86	21	1.2 (0.8-1.7)
Race					
White	369	88	359	89	1.0
Non-White	51	12	45	11	1.1 (0.7–1.7)

[a] Adjusted for all 11 risk factor variables simultaneously.
[b] Mean age: cases: 56.5 (S.D. = 10.4) years; controls: 54.1 (S.D. = 10.2) years.

ER$^-$PR$^-$, 20% were ER$^+$PR$^-$, and 13% were ER$^-$PR$^+$. Cases with missing ER/PR status were primarily those with carcinoma in situ or missing/unknown stage and were not included in this analysis. The final ER/PR analyses included 317 cases and 401 controls total, because one case in the ER$^+$PR$^+$ subtype and five controls had missing covariate data and subsequently dropped out of the models.

Table 2 presents the ORs and 95% CIs for each cancer subtype with respect to each risk factor, results from the

Table 2
Adjusted odds ratios[*] for breast cancer by combined estrogen receptor (ER) and progesterone receptor (PR) status, Yale, New Haven Hospital, Connecticut, 1994–1997

Risk factor	ER+PR+ 104/401[a]		ER−PR− 107/401[a]		ER−PR− 65/401[a]		ER−PR+ 41/401[a]		p_{Wald}[**]	ER+PR+ vs. ER−PR− 104/107[b]	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI		OR	95% CI
Age (years)											
≤50	1.0		1.0		1.0		1.0			1.0	
>50	1.1	0.6–2.0	0.9	0.5–1.7	1.2	0.5–2.6	0.8	0.3–1.9	0.87	1.1	0.5–2.5
Age at menarche (years)											
≥14	1.0		1.0		1.0		1.0			1.0	
12–13	0.9	0.5–1.6	1.2	0.7–2.0	1.0	0.5–2.0	0.9	0.4–2.3		1.4	0.5–3.7
<12	1.0	0.5–1.9	0.7	0.4–1.4	0.9	0.4–2.0	1.8	0.7–4.6	0.34	2.2	0.8–6.2
Nulliparity/age (years) at first full-term pregnancy											
<30	1.0		1.0		1.0		1.0			1.0	
≥30	0.5	0.2–1.2	1.0	0.5–1.9	2.5	1.2–5.1	0.8	0.3–2.2	0.04	0.7	0.3–1.9
Nulliparous	0.6	0.3–1.3	0.8	0.4–1.5	0.7	0.3–1.7	0.5	0.2–1.6	0.66[***]	0.7	0.3–1.9
Lifetime lactation (months)											
≥12	1.0		1.0		1.0		1.0			1.0	
1–11	0.8	0.4–1.7	0.8	0.4–1.8	0.8	0.3–1.9	1.1	0.4–3.3		1.2	0.5–3.0
Never lactated	0.8	0.4–1.5	1.1	0.6–2.1	0.9	0.4–2.0	1.0	0.4–2.8	0.96	0.8	0.4–1.9
Menopausal status											
Pre	1.0		1.0		1.0		1.0			1.0	
Post	2.4	1.1–4.9	1.3	0.7–2.6	7.2	2.4–21.7	1.6	0.6–4.6	0.04	1.9	0.8–4.8
BMI (kg/m²)											
<25.0	1.0		1.0		1.0		1.0			1.0	
25.0–29.99	0.7	0.4–1.2	1.3	0.7–2.1	0.7	0.4–1.4	1.3	0.6–2.8		0.7	0.3–1.4
≥30	1.0	0.6–19	1.3	0.7–2.3	0.8	0.4–1.8	0.9	0.3–2.3	0.54	0.7	0.3–14
Ever use of estrogen											
Never	1.0		1.0		1.0		1.0			1.0	
Ever	0.6	0.3–1.0	0.8	0.4–1.3	0.6	0.3–1.2	1.1	0.5–2.5	0.52	0.7	0.3–1.5
Alcohol intake (years)											
<1	1.0		1.0		1.0		1.0			1.0	
≥1	2.0	0.9–4.3	0.7	0.4–1.3	1.2	0.5–2.8	2.7	0.8–9.3	0.06	3.4	1.4–84
Smoking (pack years)											
Never smoked	1.0		1.0		1.0		1.0			1.0	
1–10 pack years	1.1	0.6–19	1.0	0.6–19	0.5	0.2–1.1	0	.5	0.2–1.5	1.0	0.5–2.0
>10 pack years	1.2	0.7–2.1	1.2	0.7–2.0	0.8	0.4–1.5	1.6	0.8–3.4	0.29	1.1	0.5–2.2
Family breast cancer history											
No	1.0	1.0	1.0	1.0	1.0						
Yes	1.7	1.0–2.8	1.1	0.7–19	1.5	0.8–29	1.1	0.5–2.5	0.57	1.2	0.6–24
Race											
White	1.0		1.0		1.0		1.0			1.0	
Non-White	1.1	0.5–2.2	1.6	0.9–3.0	0.8	0.3–21	1.4	0.5–3.9	0.56	0.7	0.3–1.7

[a] Number of cases/controls.
[b] Number of ER+PR+ cases vs. ER−PR− cases.
[*] All of the variables were included simultaneously in a multiple polychotomous logistic regression model. Odds ratios were adjusted for all other covariates.
[**] The Wald statistic p-value indicates the statistical significance of differences in odds ratios for a given risk factor among the four case groups.
[***] The Wald statistic p-value was calculated separately for nulliparity as a risk factor, since it is not a true category of age at first full-term pregnancy.

Wald test for heterogeneity across all four subtypes, and the case–case comparisons between the ER+PR+ and ER−PR−. Risk varied by later age (≥30 years) at first full-term pregnancy ($p = 0.04$); a significantly increased risk was found for the ER+PR− subtype (OR = 2.5; 95% CI: 1.2–5.1), while all other subtypes were not associated. Risk varied also by post-menopausal status ($p = 0.04$); significantly increased ORs were found for ER+PR+ (OR = 2.4; 95% CI: 1.1–4.9) and ER+PR− (OR = 7.2; 95% CI: 2.4–21.7) subtypes, while null effects were found for the other two subtypes. Although risk did not differ significantly across the four tumor subtypes for alcohol intake ($p = 0.06$), non-significantly increased ORs were found for ER+PR+ (OR = 2.0; 95% CI: 0.9–4.3) and ER−PR+ (OR = 2.7; 95% CI: 0.8–9.3) subtypes, while null effects were found for the other two subtypes. Additionally, in the case–case analysis, we found that cases, who consumed

alcohol for more than a year were 3.4 times more likely to have ER^+PR^+ tumors than ER^-PR^- tumors (95% CI: 1.4–8.4). No other risk factors differed significantly by ER/PR status, however, non-significantly increased ORs were found for family history of breast cancer for the ER^+PR^+ subtype (OR = 1.7; 95% CI: 1.0–2.8), and non-White race for ER^-PR^- cases (OR = 1.6; 95% CI: 0.9–3.0). A non-significantly decreased OR was found for ER^+PR^+ tumors with ever use of estrogen (OR = 0.6; 95% CI: 0.3–1.0). Risk did not vary by tumor subtype for age at diagnosis, nulliparity, lifetime lactation, BMI, and smoking. We limited the analysis to post-menopausal women only and found similar patterns (data not shown).

4. Discussion

Overall, these results indicate that some breast cancer risk factor profiles may vary by joint ER/PR status. We detected statistically significant variation in risk factor profiles among the four tumor subtypes for age at first full-term birth, menopausal status, and alcohol consumption. We detected a suggestion of variation among the four subtypes for ever use of estrogen, family history of breast cancer, and non-White race. We did not detect any variation across the subtypes for age at diagnosis, nulliparity, lactation, BMI, and smoking. We did not find a clear indication of a gradient of effect, whereby ER^+PR^+ tumors exhibited highest risks from hormone-related risk factors, ER^-PR^- tumors exhibited lowest risks, and subtypes discordant for receptor status exhibited intermediate risks.

The results from Table 1 (all cases, combined versus controls) indicated weak associations with the risk factors investigated. This led us to stratify by hormone receptor status to see whether some potential associations may have been obscured by considering breast cancer as one disease. The results from Table 2 demonstrate the necessity for considering both ER and PR status, when estimating magnitude of risk factors for breast cancer. Two risk factors, post-menopausal status and alcohol intake, varied by one of the hormone receptors, despite the other, i.e., post-menopausal status varied by ER status (ER^+) despite PR status, and alcohol intake varied by PR status (PR^+) despite ER status. However, in the case of alcohol intake, it was insightful to further categorize by both hormone receptors, since there was a significant difference between the ER^+PR^+ and ER^-PR^- subtypes. Additionally, age at first full-term pregnancy was associated only with the ER^+PR^- subtype, a finding, which would not have been revealed by stratifying by either ER status or PR status (only by stratifying on both ER and PR). Although not statistically significant, there was a suggestion of an association for ever use of estrogen (negative) and family history of breast cancer (positive) with ER^+PR^+ tumors only; non-White race was non-significantly associated with ER^-PR^- tumors only.

Despite some similarities in individual risk factor patterns, overall, the findings of our study are inconsistent with those of other studies in the literature [8,30–33,36–38,45], which evaluated a host of well-established breast cancer risk factors by joint ER/PR status. There are also inconsistencies among the previously published literature, with respect to patterns of risk among the four tumor subtypes. Regarding hormonally mediated risk factors, Potter et al. [8] found that ER^+PR^+ breast cancer showed an inverted pattern of association compared with ER^-PR^- and ER^+PR^- for most risk factors, among post-menopausal women in the Iowa Women's Study. Although we detected inverted associations for ER^+PR^+ and ER^+PR^- for age at first full-term pregnancy and heterogeneity between ER^+PR^+ and ER^-PR^- for alcohol intake, there was not a consistent pattern of inversion throughout our analysis. Potter et al. [8] also determined that it is PR^+ breast cancer, independent of ER status, which is related to endogenous hormonal risk factors, such as BMI, body fat distribution, age at menarche, and age at first birth [8]. The only risk factor we found associated with PR^+ (despite ER status) tumors was alcohol intake. Yoo et al. [33] concluded that risk factor profiles varied by PR but not by ER; our study found variation by both. Similar to the findings of Colditz et al. [31], we found heterogeneity across the four subtypes for menopausal status. However, Colditz et al. [31] did not find significant heterogeneity for alcohol use across the four ER/PR categories, while our study did (for ER^+PR^+ versus ER^-PR^-). The study by Huang et al. [32] showed a consistent association between reproductive risk factors – early age at menarche, nulliparity, late age at first full-term pregnancy and high BMI – and $ERPR^+$ breast cancer. Our study did not detect such a consistent pattern. While Giuffrida et al. [38] found increased risk for ER^+PR^+ tumors and Cotterchio et al. [34] found an increased risk for ER^-PR^- tumors with increased BMI, we did not detect any heterogeneity across the tumor subtypes for this risk factor. Similar to the findings of Enger et al. [37], we saw a positive association for the ER^+PR^+ tumor subtype with alcohol. It is difficult to compare our study results to those of Britton et al. [30] and McCredie et al. [45], since both these studies focused on younger women, and our study had very small numbers in that age group.

Regarding the non-hormonally mediated risk factor, family history, Potter et al. found it to be associated with PR^+ breast cancer only [8], a study from the same population by Tutera et al. found it to be positively associated with all subtypes except ER^+PR^- [14], Colditz et al. found it to be consistently associated with ER^+PR^+ and ER^-PR^- subtypes, and Huang et al. [32] found it associated with ER^-PR^- tumors. Our study found family history to be non-significantly associated with the ER^+PR^+ tumor subtype only.

Differences in study population could account for inconsistent findings between our study and those mentioned above. For example, the study by Yoo et al. was comprised of

all Japanese women [33], the data for the study by Huang et al. came from the Carolina Breast Cancer Study, which incorporated relatively equivalent proportions of White and Black women [32], and the study by Britton et al. included only women between the ages of 20 and 44 years [30]. Our analysis combined both pre- and post-menopausal women, of whom 88% were White.

Another important source of disparity between these studies and ours could be that different methodologies were used by each study to determine ER and PR status (i.e., positivity or negativity). In our study ER and PR levels were measured immunohistochemically at the Pathology Department of YNHH and were considered positive when their H-score was higher than 75. This is the equivalent of using 20 fmol of receptors/mg of cytosolic proteins via the dextran coated charcoal (DCC) as the cutoff between receptor positive and receptor negative [43]. One study defined ER and PR positivity as at least 10 fmol [33], while three other studies [8,31,32] established receptor status at a variety of clinical laboratories or ascertained it via medical records. Still other studies have determined positivity by the presence of at least 3 fmol/mg of protein-specific binding sites [9]. The use of an arbitrary cutoff value is a universal problem throughout the literature dealing with estrogen and progesterone receptors, and without a consensus it is difficult to make comparisons of study findings [46]. A strength of our study is that the hormone receptor status was consistently measured for the entire study population in the same laboratory, using the same methodology. As demonstrated in Table 3, the distribution of cases in the four tumor subtypes for our study are not consistent with most of those throughout the literature which have investigated the effects and heterogeneity of multiple risk factors (with the exception of Yoo et al. [29]). However, there are also inconsistencies for the distribution among most of the other studies presented in Table 3.

A strength of this study is that we used histologically confirmed non-cancer patients as controls. All controls included in the study were women histologically confirmed with normal tissue or benign breast disease without proliferation or atypical hyperplasia. Using these women as controls reduces misclassification of disease status, since we were able to ensure that none of the controls had in situ carcinoma or atypical hyperplasia. Considering both in situ breast cancer and atypical hyperplasia benign breast disease

are relatively common among seemingly healthy women, the use of population-based controls could be of concern. Current evidence suggests that any elevation in risk for breast cancer among women with BBD occurs mostly in women with proliferative lesions, in particular atypical hyperplasia [47]. Both cohort and case-controls studies have examined the association between family history, BMI, race, oral contraceptive use, and other reproductive health factors and BBD; however, there have been no clear, consistent associations [48–51]. It may be argued that the use of hospital controls might introduce a bias if the control disease is associated with the exposures of interest (i.e., all the risk factors we investigated). However, in the case of our study, this would have had a conservative effect on our results. If the control disease (benign breast disease without atypical hyperplasia) is positively associated with the risk factors under study, the use of these patients as controls would underestimate the association between these risk factors and the different subtypes of breast cancer.

A limitation of our study is the relatively small sample size when we stratify cases. The ER$^-$PR$^+$ subtype, in particular included only 41 cases, so although we found a few risk factors for which there were elevated ORs in this subtype, i.e., earlier age at menarche and alcohol intake, the confidence intervals were wide, and there was not enough power to make any definitive conclusions. There is always the possibility that multiple comparisons could have accounted for any of the significant associations we detected; however, we were more concerned with an overall pattern of variability across tumor subtypes than with specific estimates. Other potential limitations are those inherent to the case-control study design, i.e., cases' recall bias with respect to risk factors ascertained by interview after diagnosis of breast cancer.

In summary, while the results of this study did not support the hypothesis of a gradient of effect for reproductive risk factors according to receptor status, we did find variation in risk by joint ER/PR status for age at first full-term pregnancy, menopausal status, and alcohol consumption. The variability of the direction or strength of associations of specific risk factors with receptor-defined breast cancers could account for the inconsistent and weak associations seen across different studies when breast cancer is treated as a single entity. Given that certain breast cancer risk factors may vary by joint ER/PR status and given the biological and

Table 3

Distribution (%) of joint ER/PR status across studies in the literature which have investigated multiple risk factors across four tumor subtypes

Reference	Menopausal status	ER$^+$PR$^+$ (%)	ER$^-$PR$^-$ (%)	ER$^+$PR$^-$ (%)	ER$^-$PR$^+$ (%)
Potter et al. [8]	Post	68	13	16	3
Yoo et al. [33]	Pre and post	39	31	25	5
Huang et al. [32]	Pre and post	53	28	11	8
Britton et al. [30]	Mainly pre	51	30	10	10
McCredie et al. [35]	Mainly pre	53	29	6	13
Colditz et al. [31]	Post	61	20	15	4
Rusiecki (2005) – present study	Pre and post	33	34	20	13

Distribution for Cotterchio et al. not listed, since only measured ER$^+$PR$^+$ and ER$^-$PR$^-$.

clinical differences of these tumors, joint ER/PR status should be considered when evaluating risk factors for breast cancer. Our findings are intriguing and should be pursued in future studies of larger sample size, with an emphasis on using the same methodology for all cases to determine hormone receptor positivity and negativity.

References

[1] Hunter DJ, Kelsey KT. Pesticide residues and breast cancer: the harvest of a silent spring? [editorial; comment]. J Natl Cancer Inst 1993;85(8):598–9.

[2] Kelsey JL, Gammon MD. Epidemiology of breast cancer. Epidemiol Rev 1990;12:228–35.

[3] Onland-Moret NC, Peeters PH, van der Schouw YT, Grobbee DE, van Gils CH. Alcohol and endogenous sex steroid levels in postmenopausal women: a cross-sectional study. J Clin Endocrinol Metab 2004 [Epub ahead of print].

[4] Davis DL, Axelrod D, Osborne M, Telang N, Bradlow HL, Sittner E. Avoidable causes of breast cancer: the known, unknown, and the suspected, vol. 833. Annals of the New York: Academy of Sciences, 1997. p. 112–28.

[5] Davis DL, Pongsiri MJ, Wolff M. Recent developments on the avoidable causes of breast cancer vol 837, Annals of the New York: Academy of Sciences, 1997. p. 513–23.

[6] Madigan MP, Ziegler RG, Benichou J, Byrne C, Hoover RN. Proportion of breast cancer cases in the United States explained by well-established risk factors. J Natl Cancer Inst 1995;87(22):1681–5.

[7] Claus EB, Schildkraut JM, Thompson WD, Risch NJ. The genetic attributable risk of breast and ovarian cancer. Cancer 1996;77(11):2318–24.

[8] Potter JD, Cerhan JR, Sellers TA, et al. Progesterone and estrogen receptors and mammary neoplasia in the Iowa Women's Health Study: how many kinds of breast cancer are there? Cancer Epidemiol Biomarkers Prev 1995;4(4):319–26.

[9] Clark GM, Wenger CR, Beardslee S, Smith JA. How to integrate steroid hormone receptor, flow cytometric, and other prognostic information in regard to primary breast cancer. Cancer 1993;71:2157–62.

[10] Fuqua SAW. Estrogen and progesterone receptors and breast cancer. In: Harris JR, Morrow M, Lippman ME, editors. Diseases of the breast. Philadelphia: Lippincott-Raven; 1994. p. 261–71.

[11] Horwitz KB. The central role of progesterone receptors and progestational agents in the management and treatment of breast cancer. Semin Oncol 1988;15(2 Suppl. 1):14–9.

[12] Rayter Z. Steroid receptors in breast cancer. Br J Surg 1991;78(5):528–35.

[13] Wittliff JL. Steroid-hormone receptors in breast cancer. Cancer 1984;53(3 Suppl.):630–43.

[14] Tutera AM, Sellers TA, Potter JD, Drinkard CR, Wiesner GL, Folsom AR. Association between family history of cancer and breast cancer defined by estrogen and progesterone receptor status. Genet Epidemiol 1996;13(2):207–21.

[15] Stanford JL, Szklo M, Brinton LA. Estrogen receptors and breast cancer. Epidemiol Rev 1986;8:42–59.

[16] Kreiger N, King WD, Rosenberg L, Clarke EA, Palmer JR, Shapiro S. Steroid receptor status and the epidemiology of breast cancer. Ann Epidemiol 1991;1(6):513–23.

[17] Ballard-Barbash R, Griffin MR, Fisher LD, Covalciuc MA, Jiang NS. Estrogen receptors in breast cancer Association with epidemiologic risk factors. Am J Epidemiol 1986;124(1):77–84.

[18] Cooper JA, Rohan TE, Cant EL, Horsfall DJ, Tilley WD. Risk factors for breast cancer by oestrogen receptor status: a population-based case-control study. Br J Cancer 1989;59(1):119–25.

[19] Elwood JM, Godolphin W. Oestrogen receptors in breast tumours: associations with age, menopausal status and epidemiological and clinical features in 735 patients. Br J Cancer 1980;42(5):635–44.

[20] Hildreth NG, Kelsey JL, Eisenfeld AJ, LiVolsi VA, Holford TR, Fischer DB. Differences in breast cancer risk factors according to the estrogen receptor level of the tumor. J Natl Cancer Inst 1983;70(6):1027–231.

[21] Hislop TG, Coldman AJ, Elwood JM, Skippen DH, Kan L. Relationship between risk factors for breast cancer and hormonal status. Int J Epidemiol 1986;15(4):469–76.

[22] Hulka BS, Liu ET, Lininger RA. Steroid hormones and risk of breast cancer. Cancer Suppl 1994;74(3):1111–24.

[23] Lesser ML, Rosen PP, Senie RT, Duthie K, Menendez-Botet C, Schwartz MK. Estrogen and progesterone receptors in breast carcinoma: correlations with epidemiology and pathology. Cancer 1981;48(2):299–309.

[24] McTiernan A, Thomas DB, Johnson LK, Roseman D. Risk factors for estrogen receptor-rich and estrogen receptor-poor breast cancers. J Natl Cancer Inst 1986;77(4):849–54.

[25] Nasca PC, Liu S, Baptiste MS, Smith JA. Alcohol consumption and breast cancer: estrogen receptor status and histology. Am J Epidemiol 1994;140:980–8.

[26] Ruder AM, Lubin F, Wax Y, Geier A, Alfundary E, Chetrit A. Estrogen and progesterone receptors in breast cancer patients Epidemiologic characteristics and survival differences. Cancer 1989;64(1):196–202.

[27] Stanford JL, Szklo M, Boring CC, et al. A case-control study of breast cancer stratified by estrogen receptor status. Am J Epidemiol 1987;125(2):184–94.

[28] Wallace RB, Sherman BM, Bean JA. A relationship between artificial menopause, previous estrogen consumption, and estrogen receptor content of breast neoplasms: preliminary communication. Oncology 1980;37(2):111–3.

[29] Yoo KY, Tajima K, Miura S, et al. A hospital-based case-control study of breast-cancer risk factors by estrogen and progesterone receptor status. Cancer Causes Control 1993;4(1):39–44.

[30] Britton JA, Gammon MD, Schoenberg JB, et al. Risk of breast cancer classified by joint estrogen receptor and progesterone receptor status among women 20–44 years of age. Am J Epidemiol 2002;156(6):507–16.

[31] Colditz GA, Rosner BA, Chen WY, Holmes MD, Hankinson SE. Risk factors for breast cancer according to estrogen and progesterone receptor status. J Natl Cancer Inst 2004;96(3):218–28.

[32] Huang WY, Newman B, Millikan RC, Schell MJ, Hulka BS, Moorman PG. Hormone-related factors and risk of breast cancer in relation to estrogen receptor and progesterone receptor status. Am J Epidemiol 2000;151(7):703–14.

[33] Yoo KY, Tajima K, Miura S, et al. Breast cancer risk factors according to combined estrogen and progesterone receptor status: a case-control analysis. Am J Epidemiol 1997;146(4):307–14.

[34] Cotterchio M, Kreiger N, Theis B, Sloan M, Bahl S. Hormonal factors and the risk of breast cancer according to estrogen- and progesterone-receptor subgroup. Cancer Epidemiol Biomarkers Prev 2003;12:1053–60.

[35] McCredie MR, Dite GS, Southey MC, Venter DJ, Giles GG, Hopper JL. Risk factors for breast cancer in young women by oestrogen receptor and progesterone receptor status. Br J Cancer 2003;89:1661–3.

[36] Enger SM, Ross RK, Paganini-Hill A, Carpenter CL, Bernstein L. Body size, physical activity, and breast cancer hormone receptor status: results from two case-control studies. Cancer Epidemiol Biomarkers Prev 2000;9:681–7.

[37] Enger SM, Ross RK, Paganini-Hill A, Longnecker MP, Bernstein L. Alcohol consumption and breast cancer oestrogen and progesterone receptor status. Br J Cancer 1999;79(7/8):1308–14.

[38] Giuffrida D, Lupo L, La Porta GA, et al. Relation between steroid receptor status and body weight in breast cancer patients. Eur J Cancer 1992;28(1):112–5.

[39] Kushi LR, Potter JD, Bostick RM, et al. Dietary fat and risk of breast cancer according to hormone receptor status. Cancer Epidemiol Biomarkers Prev 1995;4:11–9.

[40] Chen WY, Hankinson SE, Schnitt SJ, Rosner BA, Holmes MD, Colditz GA. Association of hormone receptor therapy to estrogen and progesterone receptor status in invasive breast carcinoma. Cancer 2004;101(7):1490–500.

[41] Althuis MD, Fergenbaum JH, Garcia-Closas M, Brinton LA, Madigan MP, Sherman ME. Etiology of hormone receptor-defined breast cancer: a systematic review of the literature. Cancer Epidemiol Biomarkers Prev 2004;13(10):1558–68.

[42] Zheng T, Holford TR, Mayne ST, et al. Risk of female breast cancer associated with serum poly chlorinated biphenyls and 1,1-dichloro-2,2′-bis(*p*-chlorophenyl) ethylene. Cancer Epidemiol Biomarkers Prev 2000;9:167–74.

[43] McCarty Jr KS, Miller LS, Cox EB, Konrath J, McCarty Sr KS. Estrogen receptor analyses correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. Arch Pathol Lab Med 1985;109(8):716–21.

[44] SAS Institute. SAS/STAT User's Guide. Version 8.2 Cary, NC: SAS Institute; 2003.

[45] McCready D, Aronson KJ, Chu W, Fan W, Vesprini D, Narod SA. Breast tissue organochlorine levels and metabolic genotypes in relation to breast cancer risk Canada. Cancer Causes Control 2004;15(4):399–418.

[46] Kuo YH. Estrogen-receptor status in breast cancer. JAMA 2000;283(3):338–9.

[47] Kelsey JL, Gammon MD, John EM. Reproductive factors and breast cancer. Epidemiol Rev 1993;15(1):36–47.

[48] Brinton LA, Vessey MP, Flavel R, Yeates D. Risk factors for benign breast disease. Am J Epidemiol 1981;113(3):203–14.

[49] Friedenreich CM, Bryant HE, Alexander FE, Hugh J, Danyluk J, Page DL. Risk factors for benign proliferative breast disease. Int J Epidemiol 2000;29:637–44.

[50] Gertig DM, Stillman IE, Byrne C, et al. Association of age and reproductive factors with benign breast tissue composition. Cancer Epidemiol Biomarkers Prev 1999;8:873–9.

[51] Wang DY, Fentiman IS. Epidemiology and endocrinology of benign breast disease. Breast Cancer Res Treat 1985;6(1):5–36.